The Glymphatic-Lymphatic Continuum: Opportunities for Osteopathic Manipulative Medicine

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The brain has long been thought to lack a lymphatic drainage system. Recent studies, however, show the presence of a brain-wide paravascular system appropriately named the *glymphatic system* based on its similarity to the lymphatic system in function and its dependence on astroglial water flux. Besides the clearance of cerebrospinal fluid and interstitial fluid, the glymphatic system also facilitates the clearance of interstitial solutes such as amyloid-β and tau from the brain. As cerebrospinal fluid and interstitial fluid are cleared through the glymphatic system, eventually draining into the lymphatic vessels of the neck, this continuous fluid circuit offers a paradigm shift in osteopathic manipulative medicine. For instance, manipulation of the glymphatic-lymphatic continuum could be used to promote experimental initiatives for nonpharmacologic, noninvasive management of neurologic disorders. In the present review, the authors describe what is known about the glymphatic system and identify several osteopathic experimental strategies rooted in a mechanistic understanding of the glymphatic-lymphatic continuum.

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In osteopathic medicine, the lymphatic system plays a key role in health maintenance, with lymphatics identified as 1 of 7 care modalities of osteopathic manipulative medicine.[1] In the past couple of years, a similar system in the brain has been uncovered, termed the *glymphatic system*. The glymphatic system is currently understood to facilitate the clearance of cerebrospinal fluid (CSF), interstitial fluid (ISF), and interstitial solutes from the brain. With the right osteopathic manipulative treatment (OMT) techniques, there is potential for osteopathic physicians to provide nonpharmacologic treatment for patients with neurologic disorders. In the present article, we describe the current evidence regarding the glymphatic system and identify potential avenues for osteopathic research in this novel area.

Blood-Brain Barrier and CSF

Brain function is contingent, in part, on a vascular network that tightly regulates the movement of ions, molecules, and cells between blood and neurons. For instance, the blood-brain barrier (BBB) provides stringent control of water, interstitial solutes, and neurotransmitter flow, which allows for the transport of some molecules into the brain parenchyma.[2,3] This property is accomplished by a series of cellular and electrical gradient components that include endothelial and mural cells, basal lamina, pericytes, and astro-

cytes.[4] In addition, proteins (including claudins, occludins, and junctional adhesion molecules) further regulate the movement of ions and larger molecules across blood vessels and neural tissue. This neurovascular unit is also supported by a network of ventricles, subarachnoid spaces, cisterns, and sulci that house and transport the CSF.[5] The CSF has an important role in clearing the brain of toxins, cellular debris, soluble proteins, and metabolic products. The CSF also contains a clustering of immune cells as represented by T cells, B cells, monocytes, and dendritic cells, all of which can trigger adaptive immune responses throughout the brain.[6]

Virchow-Robin Space and Interstitial Flow

The production of CSF is not only derived from the choroid plexus but also from water flux dynamics occurring at the Virchow-Robin space (VRS), where CSF is produced.[7] The VRS is histologically defined as a space that surrounds penetrating arterioles, venules, and capillaries from the subarachnoid space into the brain parenchyma.[3,7] The flow of CSF and ISF within this pial cell–, endothelial cell–, and glial cell–lined space is referred to as *interstitial flow*[7]—a flow that does not directly communicate with the subarachnoid space. Instead, CSF interstitial flow directly drains into lymphatic channels at the base of the skull, suggesting a pathway that is equivalent to a drainage system for the clearance of waste molecules from the brain.

The Glymphatic System

The findings that interstitial bulk flow may be drained from the brain via lymphatic channels (ie, cervical lymph nodes) suggest that a conventional lymphatic system might exist in the mammalian brain. A number of recent studies[8-11] convincingly show the presence of a highly polarized lymphatic vessel system that facilitates the transport of interchangeable CSF and ISF out of the brain. The glymphatic system, appropriately named based on its functional similarity to the lymphatic system in the periphery,[11] acts as a brain-wide convective flux of CSF and ISF that is strictly dependent on isoform water channel aquaporin-4 (AQP4) expressed in astrocyte foot processes.[12] It is now clear that aquaporin proteins regulate the movement of water across biological membranes, including astrocytic membranes of the VRS and the BBB.[3] Thus, CSF and ISF, both solute-bearing liquids filtered from the blood, enter the VRS along penetrating arterioles, where they diffuse primarily through AQP4 channels, ultimately emptying into the jugular vein.[3,11] This glymphatic hydrodynamic process is bidirectional in terms of communication flux and is driven, in part, by respiratory and cardiac pressure pulsations.[8] These latter findings suggest that arterial pulsatility drives clearance of interstitial solutes and fluids from the brain (*Figure 1*). Indeed, inspiratory thoracic pressure reduction is the major regulator of human CSF flow, as demonstrated with real-time magnetic resonance (MR) imaging at high spatial and temporal resolutions.[13]

Lymphatic Vessels in the Brain and the Glymphatic System

The presence of a glymphatic system is further supported by the structural identification of lymphatic vessels in the mouse brain. Lymphatic endothelial cells localized to the meninges (eg, dura mater) appear to be capable of carrying fluid from the CSF and immune cells to deep cervical lymph nodes.[14] The presence of a functional and classical lymphatic unit within the mammalian brain may explain, in part, how immune cells (eg, T cells, dendritic cells) enter and leave the central nervous system and suggest that traditional textbook concepts of brain tolerance and the immune privilege of the brain should be revisited.[14]

In general, the anatomical characterization of lymphatic vessels and a glymphatic system that promotes the elimination of soluble proteins from the brain may be

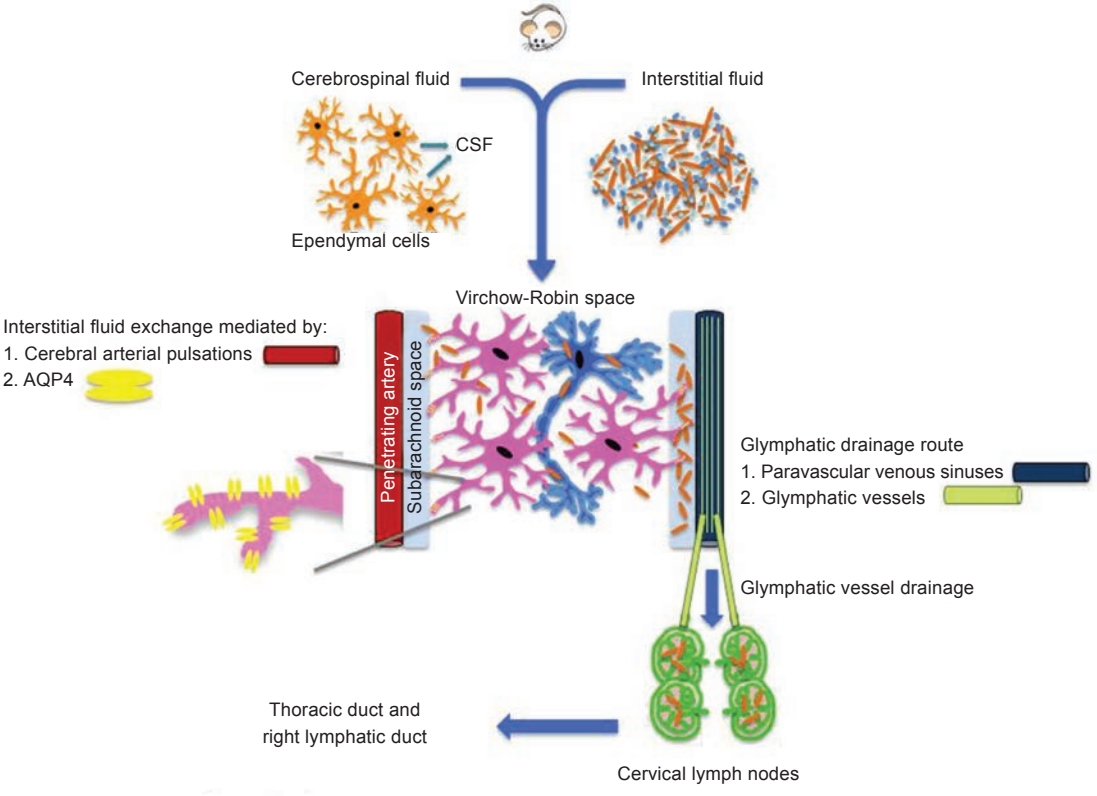

Figure 1.
Schematic microscopic diagram of the glymphatic-lymphatic continuum within the mouse brain. Cerebrospinal fluid (CSF) synthesized by ependymal cells of the ventricles enters the Virchow-Robin space (VRS) along paravascular arteries into the brain parenchyma. Interstitial fluid exchange along the VRS is mediated by cerebral arterial pulsations as well as aquaporin-4 (AQP4) water transport. The flow of CSF across the VRS deposits interstitial solutes such as amyloid-β and tau into a paravascular venous sinus harboring glymphatic vessels. These interstitial solutes enter glymphatic vessels within dural sinuses and drain into cervical lymph nodes, bilaterally. The left- and right-sided cervical lymph nodes drain interstitial solutes into lymphatic channels, which then enter systemic circulation via the thoracic duct and right lymphatic duct, respectively.

relevant for neurodegenerative diseases such as Alzheimer disease (AD), which is characterized by the deposition of amyloid plaques and tau tangles.[15,16] Under some circumstances, these toxic, aggregation-prone proteins accumulate in sufficient quantity within the brain as well as within the vascular wall of arteries and arterioles to initiate clinical features of AD. Amyloid-β appears to be rapidly cleared along the glymphatic paravenous efflux system of the mouse brain, and genetic knock-out of the gene encoding AQP4 drastically reduces the clearance of interstitial soluble amyloid-β.[11,12] Thus, a role of the glymphatic system is to assist in the clearance of unwanted and potentially noxious proteins, and seeking treatments that enhance drainage of amyloid-β and microtubule-associated tau is rational (*Figure 2*).

Osteopathic Manipulative Medicine and the Glymphatic System

Building on the knowledge gathered from existing studies,[7-12,14] preventing the deposition of aggregation-prone proteins and their downstream effects or, conversely, accelerating the clearance of amyloid-β and tau from the brain may be effective approaches to therapy. For instance, osteopathic manipulative medicine (OMM) could be a practical option for promoting lymphatic drainage, as several studies[17-19] have provided important proof of principle (*Figure 3*).

Osteopathic manipulative treatment applied to the glymphatic system would have the same 4 goals as OMT applied to the lymphatic system: (1) open myofascial transition areas, (2) maximize diaphragmatic movement, (3) augment lymphatic flow, and (4) mobilize fluid in the lymphaticovenous system.[20] The *Table* identifies 10 OMT techniques and the tissues targeted. Their mechanisms of action can be described as follows:

- Thoracic outlet release is an essential technique to open myofascial restrictions because all terminal drainages pass through the cervical thoracic diaphragm.[20] A variety of osteopathic cranial manipulative medicine techniques may be used to relieve restrictions to glymphatic drainage from the head.
- The V-spread improves articulation between the temporal and occipital bones and thus can remove restriction in the jugular foramen, where the internal jugular vein and the glymphatic system pass.[21,22]
- Jugulodigastric release and clavicle muscle energy can be performed to manage restrictions between the head and lymphatic duct.[20]
- Techniques such as the parietal lift free restrictions in the parietal bone and can relieve distortions of tension in the attached dural venous sinuses.[23]

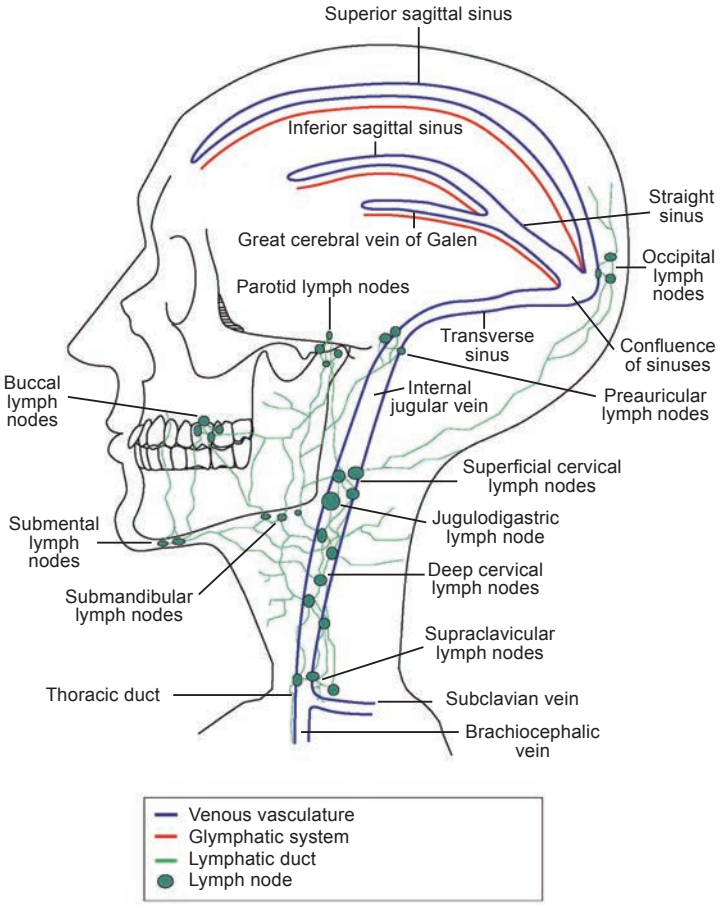

Figure 2.
Schematic macroscopic diagram depicts the glymphatic-lymphatic continuum system within the human brain. Interstitial soluble molecules such as amyloid-β are removed from the brain and received in the paravascular space along the intracranial sinuses. The superior sagittal, inferior sagittal, and straight sinuses meet at the confluence of sinuses and then travel primarily to the internal jugular vein. From here, interstitial solutes residing within the interstitial fluid are deposited within the deep cervical lymph nodes where they combine with the lymph nodes to follow a well-established lymphatic drainage system. Lymph enters the thoracic duct and right lymphatic duct, which then drain into the right and left subclavian veins. Ultimately, passage of interstitial solutes reenter systemic circulation through the superior vena cava. The 2015 finding of lymphatic vessels lining the dural sinuses and connecting to the deep cervical lymph nodes adds validation of a novel pathway for cerebrospinal fluid drainage in the mammalian brain.[14] Additional lymph nodes are presented in the figure (eg, parotid lymph nodes). However, their relationship with the glymphatic system is unclear.

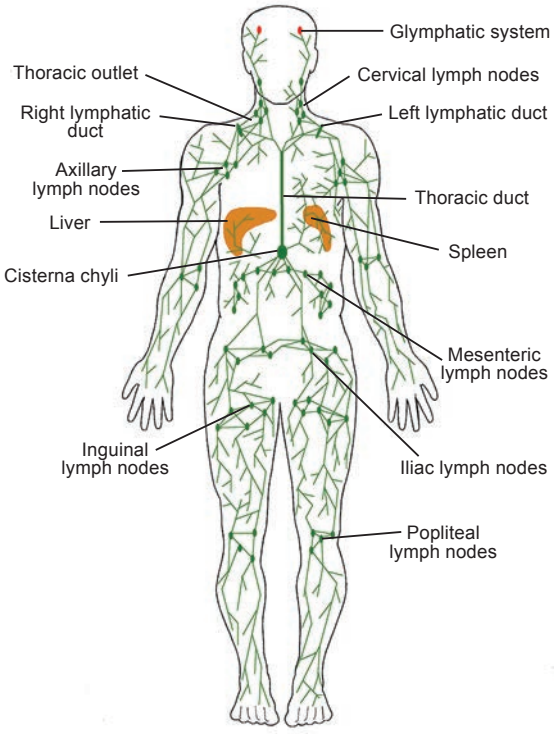

Figure 3.
Schematic diagram depicting the general locations of key lymphatic tissues as well as the proposed glymphatic system in the brain.

- Venous sinus drainage decreases congestion and augments flow through venous sinuses.[22] Osteopathic cranial manipulative medicine may also be used to affect the nervous system.
- Compression of the fourth ventricle enhances the primary respiratory mechanism, affects the exchange of fluids in the body, and alters sleep latency and sympathetic nerve activity.[24] After opening myofascial restrictions to glymphatic flow back into the general circulatory system, OMM can be used to improve the function of the body's largest fluid pump, the thoracolumbar respiratory diaphragm.
- Doming of the diaphragm is used to return proper shape to this diaphragm, thus improving respiratory motion.[17]
- Drainage along the sternocleidomastoid muscle can improve lymphatic flow through the deep cervical chain of lymph nodes.[20]
- Thoracic pump augments lymph flow through the right lymphatic and thoracic ducts.[18,19]

These OMT techniques emphasize and parallel the previously mentioned considerations, including altering brain arousal, augmenting drainage by means of body posture, and improving respiratory patterns. By addressing restrictions throughout the body, posture and respiration is improved and lymphatic drainage is enhanced.

Several experimental variables exist for the management of neurologic disorders on the basis of lymphatic drainage of CSF and ISF. To expedite clearance of waste, including interstitial soluble amyloid-β from the brain, OMT could be used with the following considerations:

- **Brain's arousal level:** Glymphatic transport activity (ie, CSF-ISF exchange in the brain) is enhanced during sleep or anesthesia and suppressed during wakefulness.[11,25] Experimental evidence indicates that brain interstitial space volume expands significantly during deep-wave sleep.[25] In addition, these OMT techniques target the cranial bones, which would indirectly affect the meninges with the potential of altering brain arousal level and glymphatic transport activity.
- **Body posture:** Glymphatic transport activity is most efficient in the right lateral position compared with the supine or prone positions.[26] Body position is known to influence sympathetic tone, with sympathetic tone being lower in the right lateral position compared with that in the left lateral position.[27] Body position is also known to affect respiratory function, particularly during the night sleep cycle.[28,29]

- **Respiration patterns:** Flow of the CSF is substantially increased during inspiration, particularly when performed during forced breathing.[13] It is conceivable, then, that inspiratory thoracic pressure may also contribute to glymphatic transport activity.

We suggest that these 3 independent variables be considered in future OMT research with regard to glymphatic removal of amyloid-β and other aggregation-prone proteins (eg, tau, α-synuclein) that are threats to cell function and viability. Patients with neurodegenerative disorders such as AD, Pick disease, progressive supranuclear palsy, amyotrophic lateral sclerosis, parkinsonism-dementia complex, and chronic traumatic encephalopathy (a degenerative condition linked to repeated head injuries) are prime candidates for OMT, as these disorders all share a conspicuous common feature of the same pathologic process: deposition of abnormal proteins in the brain.

Experimental Study: An Example

One potential study that could provide sufficient evidence of a clinical benefit of OMT would be to test amyloid-β turnover in healthy individuals. The working hypothesis here would be that as people age, amyloid-β turnover (the dependent variable) slows down and OMT improves protein turnover kinetics. To test this hypothesis, participants of different ages (eg, 18-68 years) would be infused with an isotope-labeled version of the amino acid leucine and would be tracked for newly synthesized amyloid-β in the blood and CSF for 24 to 48 hours after OMT. Appropriate control participants would be included. This study would be a preventive treatment trial for neurodegenerative diseases (see question 3 in the following section).

This example highlights the transformative potential cross-talk between the neurosciences and OMM. Clinically significant advances in the management of neurologic disorders using current OMM techniques will depend, in part, on 3 important questions raised in the following section.

Table.
Osteopathic Manipulative Treatment Techniques to Promote Lymphatic Drainage

Technique	Tissue Targeted
Thoracic outlet release	Cervical thoracic diaphragm
V-spread	Occipitomastoid suture and jugular foramen
Jugulodigastric release	Cervical lymph nodes
Clavicle muscle energy	Cervical lymph nodes
Parietal lift	Parietal bone and tentorium cerebelli
Venous sinus drainage	Occipital transverse, straight, superior sagittal, saggital (metopic suture) sinuses
CV-4	Floor of the fourth ventricle
Doming of the diaphragm	Thoracic diaphragm
Drainage along the SCM	Cervical lymph nodes
Thoracic pump	Rib cage, thoracic duct, and right lymphatic duct

Abbreviations: CV-4, compression of the fourth ventricle; SCM, sternocleidomastoid muscle.

3 Important Questions for OMM

1. How can the glymphatic system be harnessed to manage certain neurologic disorders with OMT?

Why It Matters: The pharmacologic pipeline behind most neurologic disorders is sparse. In addition, no currently available drug treatments for most neurologic disorders address the underlying pathologic process. Osteopathic manipulative medicine could help bridge this gap by providing nonpharmacologic, noninvasive treatments for patients with diseases that are characterized by the accumulation of clumps of proteins in the brain.

What We Know: The glymphatic system is thought to expedite clearance of interstitial soluble proteins, including amyloid-β, from the brain. Although most of the described work has been done on mouse models, mice share important anatomical and physiologic similarities with humans.

Next Steps: Lymphatic drainage is not well understood. Researchers must move toward a mechanistic understanding of lymphatic and now glymphatic transport activity physiology, with a greater role for MR imaging and CSF flow measurements.

2. If the glymphatic system can be targeted with OMT, can we develop a glymphatic diagnostic test based on specific OMT techniques?

Why It Matters: Tests are urgently needed that can reliably and specifically predict which patients are going to develop a neurologic disorder before clinically significant cell damage occurs.

What We Know: A number of risk factors might contribute to the development of certain neurologic disorders. For example, apolipoprotein E-4 (APOE-4) is a genetic risk factor for the development of AD. An inexpensive diagnostic test that is simple to apply across multiple neurologic diseases is needed.

Next Steps: In principle, MR imaging can be used to see changes in CSF flow in the brain in living patients before and after OMT. Elevated levels of amyloid-β and tau can be detected in the CSF and blood of patients with mild forms of neurodegenerative diseases. It would be of interest to test whether OMT could facilitate drainage of the interstitial soluble molecules and produce a measurable health benefit.

3. Can OMT, by targeting the glymphatic system, be used as a preventive tool?

Why It Matters: One of the goals of medicine today is to develop novel therapies that either prevent or delay onset of disease.[30-32] For this goal to be reached, it is important to validate the usefulness and predictability of the glymphatic system in human clinical trials. As such, developing appropriate OMT techniques that modify disease progression merits further investigation.

What We Know: To our knowledge, there are few OMT techniques currently used to slow or even halt the progression of neurologic disorders. If OMT techniques were to be developed, a noninvasive treatment could be useful for managing disease at an early stage.

Next Steps: For practical and ethical reasons, many experimental tests cannot be used in humans. Osteopathic manipulative treatment could easily be applied to minimize the load of potentially noxious proteins in the brain through manipulation of the glympathic-lymphatic continuum.

Conclusion

The glymphatic system represents a novel area for research in the osteopathic medical profession. Increased understanding of the glymphatic-lymphatic continuum may allow the development of rational, effective OMT techniques for neurologic disorders.

Author Contributions

Student Doctors Hitscherich, Smith, Cuoco, and Ruvolo and Drs Mancini and Leheste provided substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; all authors drafted the article or revised it critically for important intellectual content; Dr Torres gave final approval of the version of the article to be published; and all authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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